```
FILE 'HOME' ENTERED AT 15:59:36 ON 25 MAR 2008
=> fil .bec
COST IN U.S. DOLLARS
                                               SINCE FILE
                                                              TOTAL
                                                    ENTRY
                                                            SESSION
FULL ESTIMATED COST
                                                     0.21
                                                                0.21
FILES 'MEDLINE, SCISEARCH, LIFESCI, BIOTECHDS, BIOSIS, EMBASE, HCAPLUS, NTIS,
      ESBIOBASE, BIOTECHNO, WPIDS' ENTERED AT 16:00:00 ON 25 MAR 2008
ALL COPYRIGHTS AND RESTRICTIONS APPLY. SEE HELP USAGETERMS FOR DETAILS.
11 FILES IN THE FILE LIST
=> s xylanase?(10a)(thermophil? or hyperthermo? or thermotol? or thermostab?)
FILE 'MEDLINE'
         1975 XYLANASE?
        10724 THERMOPHIL?
         2596 HYPERTHERMO?
         2269 THERMOTOL?
         7642 THERMOSTAB?
L1
          218 XYLANASE? (10A) (THERMOPHIL? OR HYPERTHERMO? OR THERMOTOL? OR THER
FILE 'SCISEARCH'
         4484 XYLANASE?
        17994 THERMOPHIL?
         3526 HYPERTHERMO?
         4055 THERMOTOL?
        10828 THERMOSTAB?
          471 XYLANASE? (10A) (THERMOPHIL? OR HYPERTHERMO? OR THERMOTOL? OR THER
L2
              MOSTAB?)
FILE 'LIFESCI'
         2176 XYLANASE?
         9999 THERMOPHIL?
         1857 HYPERTHERMO?
         1397 THERMOTOL?
         4742 THERMOSTAB?
T. 3
          292 XYLANASE?(10A)(THERMOPHIL? OR HYPERTHERMO? OR THERMOTOL? OR THER
              MOSTAB?)
FILE 'BIOTECHDS'
         2828 XYLANASE?
         6250 THERMOPHIL?
          512 HYPERTHERMO?
          527 THERMOTOL?
         7396 THERMOSTAB?
          387 XYLANASE?(10A)(THERMOPHIL? OR HYPERTHERMO? OR THERMOTOL? OR THER
L4
              MOSTAB?)
FILE 'BIOSIS'
         4781 XYLANASE?
        24303 THERMOPHIL?
         3060 HYPERTHERMO?
         3852 THERMOTOL?
        13144 THERMOSTAB?
1.5
          428 XYLANASE? (10A) (THERMOPHIL? OR HYPERTHERMO? OR THERMOTOL? OR THER
              MOSTAB?)
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FILE 'EMBASE'
          1930 XYLANASE?
         11044 THERMOPHIL?
          2355 HYPERTHERMO?
          1951 THERMOTOL?
         15150 THERMOSTAB?
L6
           286 XYLANASE?(10A)(THERMOPHIL? OR HYPERTHERMO? OR THERMOTOL? OR THER
               MOSTAB?)
FILE 'HCAPLUS'
          8310 XYLANASE?
         23115 THERMOPHIL?
          3360 HYPERTHERMO?
          3075 THERMOTOL?
         22607 THERMOSTAB?
           699 XYLANASE?(10A)(THERMOPHIL? OR HYPERTHERMO? OR THERMOTOL? OR THER
               MOSTAB?)
FILE 'NTIS'
            51 XYLANASE?
           499 THERMOPHIL?
            33 HYPERTHERMO?
            45 THERMOTOL?
           201 THERMOSTAB?
L8
             7 XYLANASE?(10A)(THERMOPHIL? OR HYPERTHERMO? OR THERMOTOL? OR THER
               MOSTAB?)
FILE 'ESBIOBASE'
          2115 XYLANASE?
          7560 THERMOPHIL?
          2287 HYPERTHERMO?
          1528 THERMOTOL?
          4651 THERMOSTAB?
1.9
           286 XYLANASE?(10A)(THERMOPHIL? OR HYPERTHERMO? OR THERMOTOL? OR THER
               MOSTAB?)
FILE 'BIOTECHNO'
          1496 XYLANASE?
          6914 THERMOPHIL?
          1350 HYPERTHERMO?
          1034 THERMOTOL?
          6565 THERMOSTAB?
L10
           215 XYLANASE? (10A) (THERMOPHIL? OR HYPERTHERMO? OR THERMOTOL? OR THER
               MOSTAB?)
FILE 'WPIDS'
          1323 XYLANASE?
          3127 THERMOPHIL?
           146 HYPERTHERMO?
           190 THERMOTOL?
          6020 THERMOSTAB?
L11
            47 XYLANASE? (10A) (THERMOPHIL? OR HYPERTHERMO? OR THERMOTOL? OR THER
               MOSTAB?)
TOTAL FOR ALL FILES
L12
          3336 XYLANASE?(10A)(THERMOPHIL? OR HYPERTHERMO? OR THERMOTOL? OR THER
               MOSTAB?)
=> s xylanase?(10a)alkali?
FILE 'MEDLINE'
          1975 XYLANASE?
```

104041 ALKALI?

```
L13
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FILE 'SCISEARCH'
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4484 XYLANASE?

159888 ALKALI?

L14 190 XYLANASE?(10A)ALKALI?

#### FILE 'LIFESCI'

2176 XYLANASE?

26497 ALKALI?

L15 119 XYLANASE? (10A) ALKALI?

# FILE 'BIOTECHDS'

2828 XYLANASE?

10338 ALKALI?

L16 165 XYLANASE? (10A) ALKALI?

#### FILE 'BIOSIS'

4781 XYLANASE?

147389 ALKALI?

160 XYLANASE? (10A) ALKALI?

#### FILE 'EMBASE'

1930 XYLANASE?

89940 ALKALI?

L18 102 XYLANASE? (10A) ALKALI?

#### FILE 'HCAPLUS'

8310 XYLANASE?

577402 ALKALI?

434990 ALK 25638 ALKY

872995 ALKALI?

(ALKALI? OR ALK OR ALKY)

L19 368 XYLANASE? (10A) ALKALI?

## FILE 'NTIS'

51 XYLANASE?

13212 ALKALI?

L20 2 XYLANASE?(10A)ALKALI?

#### FILE 'ESBIOBASE'

2115 XYLANASE?

30444 ALKALI?

L21 129 XYLANASE?(10A)ALKALI?

#### FILE 'BIOTECHNO'

1496 XYLANASE?

21300 ALKALI?

83 XYLANASE? (10A) ALKALI?

FILE 'WPIDS'

1323 XYLANASE? 309504 ALKALI?

L23 76 XYLANASE?(10A)ALKALI?

# TOTAL FOR ALL FILES

L24 1470 XYLANASE? (10A) ALKALI?

### => s 112 and 124

FILE 'MEDLINE'

L25 21 L1 AND L13

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FILE 'SCISEARCH'
          64 L2 AND L14
1.26
FILE 'LIFESCI'
L27
          32 L3 AND L15
FILE 'BIOTECHDS'
L28
          46 L4 AND L16
FILE 'BIOSIS'
L29
           44 L5 AND L17
FILE 'EMBASE'
L30
          30 L6 AND L18
FILE 'HCAPLUS'
L31
           84 L7 AND L19
FILE 'NTIS'
L32
           0 L8 AND L20
FILE 'ESBIOBASE'
L33
           41 L9 AND L21
FILE 'BIOTECHNO'
          25 L10 AND L22
FILE 'WPIDS'
           6 L11 AND L23
L35
TOTAL FOR ALL FILES
L36
         393 L12 AND L24
=> s 136 not 2004-2008/py
FILE 'MEDLINE'
      2738674 2004-2008/PY
                (20040000-20089999/PY)
L37
           13 L25 NOT 2004-2008/PY
FILE 'SCISEARCH'
       5082921 2004-2008/PY
                (20040000-20089999/PY)
L38
          42 L26 NOT 2004-2008/PY
FILE 'LIFESCI'
       596361 2004-2008/PY
L39
          20 L27 NOT 2004-2008/PY
FILE 'BIOTECHDS'
       107093 2004-2008/PY
           33 L28 NOT 2004-2008/PY
L40
FILE 'BIOSIS'
       2353565 2004-2008/PY
           28 L29 NOT 2004-2008/PY
FILE 'EMBASE'
       2396479 2004-2008/PY
          18 L30 NOT 2004-2008/PY
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FILE 'HCAPLUS'

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5502727 2004-2008/PY
1.43
           51 L31 NOT 2004-2008/PY
FILE 'NTIS'
        66049 2004-2008/PY
             0 L32 NOT 2004-2008/PY
L44
FILE 'ESBIOBASE'
       1370575 2004-2008/PY
L45
            26 L33 NOT 2004-2008/PY
FILE 'BIOTECHNO'
           586 2004-2008/PY
1.46
            25 L34 NOT 2004-2008/PY
FILE 'WPIDS'
      4627257 2004-2008/PY
L47
            3 L35 NOT 2004-2008/PY
TOTAL FOR ALL FILES
L48
           259 L36 NOT 2004-2008/PY
```

=> d tot

=> dup rem 148

- L49 ANSWER 1 OF 87 SCISEARCH COPYRIGHT (c) 2008 The Thomson Corporation on STN DUPLICATE 1
- ΤI Thermostable and alkaline-tolerant microbial cellulase-free xylanases produced from agricultural wastes and the properties required for use in pulp bleaching bioprocesses: a review
- PROCESS BIOCHEMISTRY, (30 APR 2003) Vol. 38, No. 9, pp. 1327-1340. SO ISSN: 0032-9592.

87 DUP REM L48 (172 DUPLICATES REMOVED)

PROCESSING COMPLETED FOR L48

- AII Techapun C; Poosaran N; Watanabe M; Sasaki K (Reprint)
- AN 2003:543080 SCISEARCH
- L49 ANSWER 2 OF 87 SCISEARCH COPYRIGHT (c) 2008 The Thomson Corporation on STN
- Production of xylanases from rice bran by Streptomyces actuosus A-151
- SO ENZYME AND MICROBIAL TECHNOLOGY, (2 DEC 2003) Vol. 33, No. 7, pp. 917-925. ISSN: 0141-0229.
- ΑU Wang S L (Reprint); Yen Y H; Shih I L; Chang A C; Chang W T; Wu W C; Chai Y D
- 2003:1069584 SCISEARCH AN
- L49 ANSWER 3 OF 87 BIOSIS COPYRIGHT (c) 2008 The Thomson Corporation on STN ΤI Characterization of a family 11 xylanase from Bacillus subtillis B230 used
- for paper bleaching. Acta Crystallographica Section D Biological Crystallography, (April 2003)
- Vol. 59, No. 4, pp. 627-636. print. ISSN: 0907-4449.
- AIT Oakley, Aaron J.; Heinrich, Tatjana; Thompson, Colin A.; Wilce, Matthew C. J. [Reprint Author]
  - 2003:253581 BIOSIS
- L49 ANSWER 4 OF 87 SCISEARCH COPYRIGHT (c) 2008 The Thomson Corporation on
- Effect of Bacillus circulans D1 thermostable xylanase on biobleaching of eucalyptus kraft pulp
- SO APPLIED BIOCHEMISTRY AND BIOTECHNOLOGY, (SPR 2003) Vol. 105, pp. 393-401.

ISSN: 0273-2289.

- AU Bocchini D A; Damiano V B; Gomes E; Da Silva A (Reprint)
- AN 2003:398916 SCISEARCH
- L49 ANSWER 5 OF 87 HCAPLUS COPYRIGHT 2008 ACS on STN
- TI Application of thermotolerant and alkaline-tolerant xylanase produced from agricultural wastes for pulp bleaching

process and reductions of amounts of chlorine compounds in wastewater Mizu Shori Gijutsu (2003), 44(6), 271-278

- CODEN: MSYGAO; ISSN: 0026-7015
- AU Sasaki, Ken; Techapun, Charin; Poosaran, Niyatat
- AN 2003:487363 HCAPLUS
- DN 139:135090

SO

- L49 ANSWER 6 OF 87 HCAPLUS COPYRIGHT 2008 ACS on STN
- TI Advances in alkaline and thermophilic
- xvlanases
- SO Zhongguo Shengwu Gongcheng Zazhi (2003), 23(7), 72-75, 88 CODEN: ZSGZAW; ISSN: 1671-8135
- AU Xie, Fuhong; Li, Wenpeng; Zhang, Kegin
- AN 2004:313894 HCAPLUS
- DN 141:67100
- L49 ANSWER 7 OF 87 SCISEARCH COPYRIGHT (c) 2008 The Thomson Corporation on STN DUPLICATE 2
- TI Overproduction of an alkali- and thermo-stable xylanase
- in tobacco chloroplasts and efficient recovery of the enzyme SO MOLECULAR BREEDING, (JAN 2003) Vol. 11, No. 1, pp. 59-67.
- ISSN: 1380-3743. AU Leelavathi S; Gupta N; Maiti S; Ghosh A; Reddy V S (Reprint)
- AN 2003:142329 SCISEARCH
- L49 ANSWER 8 OF 87 BIOTECHDS COPYRIGHT 2008 THE THOMSON CORP. on STN
- TI Bleaching of chemical pulp involves, exposing chemical pulp to acidic bleaching stage to produce partially bleached pulp and treating with thermophilic, alkalophilic xylanase in alkaline extraction stage at preset condition;
  - pulp bleaching using recombinant enzyme
- AU TOLAN J; POPOVICÍ C; FOODY P J
- AN 2003-01501 BIOTECHDS
- PI WO 2002052100 4 Jul 2002
- L49 ANSWER 9 OF 87 BIOTECHDS COPYRIGHT 2008 THE THOMSON CORP. on STN
- TI Novel xylanase activity protein, useful in bleaching process of pulp and in food and animal feed industry, has enhanced thermostability and alkalophilicity;
  - recombinant enzyme production via plasmid expression useful for animal feedstuff
- AU BENTZIEN J; DAHIYAT B
- AN 2003-01486 BIOTECHDS
- PI WO 2002038746 16 May 2002
- L49 ANSWER 10 OF 87 MEDLINE on STN DUPLICATE 3
- TI Thermostable and alkaline-tolerant cellulase-free xylanase produced by thermotolerant Streptomyces sp. Abi06.
- SO Journal of bioscience and bioengineering, (2002) Vol. 93, No. 4, pp. 431-3.
  - Journal code: 100888800. ISSN: 1389-1723.
- AU Techapun Charin; Charoenrat Thanakorn; Poosaran Naiyatat; Watanabe Masanori; Sasak Ken
- AN 2005557533 MEDLINE

- L49 ANSWER 11 OF 87 SCISEARCH COPYRIGHT (c) 2008 The Thomson Corporation on STN
- Employing chimeric xylanases to identify regions of an alkaline xylanase participating in enzyme activity at
- JOURNAL OF BIOSCIENCE AND BIOENGINEERING, (NOV 2002) Vol. 94, No. 5, pp. SO 395-400. ISSN: 1389-1723.
- AIT Nishimoto M; Kitaoka M (Reprint); Havashi K
- AN 2003:96848 SCISEARCH
- L49 ANSWER 12 OF 87 SCISEARCH COPYRIGHT (c) 2008 The Thomson Corporation on
- ΤI Enzymatic properties of a neutral endo-1,3(4)-beta-xylanase Xyl II from Bacillus subtilis
- JOURNAL OF BIOTECHNOLOGY, (11 APR 2002) Vol. 94, No. 3, pp. 265-275. SO ISSN: 0168-1656.
- ΔII Sa-Pereira P (Reprint); Costa-Ferreira M; Aires-Barros M R
- AN 2002:276532 SCISEARCH
- L49 ANSWER 13 OF 87 SCISEARCH COPYRIGHT (c) 2008 The Thomson Corporation on STN DUPLICATE 4
- ΤI Cellulase-free thermostable alkaline xylanase
- from thermophilic and alkalophilic Bacillus sp JB-99
- JOURNAL OF MICROBIOLOGY AND BIOTECHNOLOGY, (FEB 2002) Vol. 12, No. 1, pp. 153-156.
  - ISSN: 1017-7825.
- AΠ Johnvesly B; Virupakshi S; Patil G N; Ramalingam; Naik G R (Reprint)
- ΔN 2002:241601 SCISEARCH
- L49 ANSWER 14 OF 87 MEDLINE on STN
- Engineering of multiple arginines into the Ser/Thr surface of Trichoderma TΤ reesei endo-1,4-beta-xylanase II increases the thermotolerance and shifts the pH optimum towards alkaline

DUPLICATE 5

- рH. SO Protein engineering, (2002 Feb) Vol. 15, No. 2, pp. 141-5.
- Journal code: 8801484. ISSN: 0269-2139.
- AΠ Turunen Ossi; Vuorio Mika; Fenel Fred; Leisola Matti
- AN 2002184634 MEDITNE
- L49 ANSWER 15 OF 87 MEDLINE on STN DUPLICATE 6
- ΤI The endoxylanases from family 11: computer analysis of protein sequences reveals important structural and phylogenetic relationships.
- SO Journal of biotechnology, (2002 May 9) Vol. 95, No. 2, pp. 109-31.
- Journal code: 8411927. ISSN: 0168-1656. Sapag Amalia; Wouters Johan; Lambert Christophe; de Ioannes Pablo; AII
- Evzaguirre Jaime; Depiereux Eric
- 2002179500 MEDLINE ΔN
- L49 ANSWER 16 OF 87 SCISEARCH COPYRIGHT (c) 2008 The Thomson Corporation on DUPLICATE 7
- Optimization of thermostable and alkaline-tolerant cellulase-free xylanase production from agricultural waste by thermotolerant Streptomyces sp Ab106, using the central composite experimental design
- SO BIOCHEMICAL ENGINEERING JOURNAL, (NOV 2002) Vol. 12, No. 2, pp. 99-105. ISSN: 1369-703X.
- AΠ Techapun C; Charoenrat T; Watanabe M; Sasaki K (Reprint); Poosaran N 2002:870007 SCISEARCH AN
- L49 ANSWER 17 OF 87 HCAPLUS COPYRIGHT 2008 ACS on STN

- Computer directed High-Throughput Screening for improved enzymatic catalysis: Towards the rationale design of a thermostable, alkaliphilic xylanase
- Abstracts of Papers, 223rd ACS National Meeting, Orlando, FL, United States, April 7-11, 2002 (2002), CELL-092 Publisher: American Chemical Society, Washington, D. C. CODEN: 69CKQP
- AU Bentzien, Jorg; Hayes, Robert; Muchhal, Umesh; O'Keefe, Donald; Dahiyat, Bassil
- AN 2002:186502 HCAPLUS
- L49 ANSWER 18 OF 87 MEDLINE on STN
  - DUPLICATE 8
- ΤI Properties and application of a partially purified alkaline xylanase from an alkalophilic fungus Aspergillus nidulans KK-99. Bioresource technology, (2002 Oct) Vol. 85, No. 1, pp. 39-42. SO
- Journal code: 9889523. ISSN: 0960-8524. Taneja Kavita; Gupta Saurabh; Kuhad Ramesh Chander AIT
- AN 2002397711 MEDLINE
- L49 ANSWER 19 OF 87 HCAPLUS COPYRIGHT 2008 ACS on STN
- In-situ solid-state fermentation and utilization of xylanase in pulp bleaching
- SO Abstracts of Papers, 223rd ACS National Meeting, Orlando, FL, United States, April 7-11, 2002 (2002), CELL-039 Publisher: American Chemical Society, Washington, D. C. CODEN: 69CKQP
- AII Szendefy, Judit; Szakacs, George; Christov, Lew
- 2002:186449 HCAPLUS AN
- L49 ANSWER 20 OF 87 HCAPLUS COPYRIGHT 2008 ACS on STN
- Use of biological agents for pulping and bleaching in pulp and paper industry
- IPPTA (2002), 14(4), 29-31
- CODEN: IPPTDO; ISSN: 0379-5462
- ΑU Sapre, M.; Jha, H.; Patil, M. B.; Dhake, J. D. 2003:27382 HCAPLUS
- AN
- DN 138:370507
- L49 ANSWER 21 OF 87 HCAPLUS COPYRIGHT 2008 ACS on STN
- Recombinant Bacillus and fermentation process for preparation of ΤI thermostable alkali-stable xylanase SO Indian, 35 pp.
- CODEN: INXXAP
- IN Gupta, Naveen; Ghosh, Amit
- AN 2004:869800 HCAPLUS
- DN 141:313041
- PATENT NO. KIND DATE APPLICATION NO. DATE ------------------------PΤ IN 185709 A1 20010414 IN 1996-DE2308 19961025
- L49 ANSWER 22 OF 87 SCISEARCH COPYRIGHT (c) 2008 The Thomson Corporation on STN
- Directed evolution to produce an alkalophilic variant from a
- Neocallimastix patriciarum xvlanase
- CANADIAN JOURNAL OF MICROBIOLOGY, (DEC 2001) Vol. 47, No. 12, pp. 1088-1094.
  - ISSN: 0008-4166.
- Chen Y L; Tang T Y; Cheng K J (Reprint) AII
- AN 2002:32328 SCISEARCH
- L49 ANSWER 23 OF 87 WPIDS COPYRIGHT 2008 THE THOMSON CORP on STN
- TI Non naturally occurring XA protein with enhanced thermophilicity

- , alkalophilicity or thermostability relative to the naturally occurring Bacillus circulans xylanase is used in an agent for bleaching pulp
- PI WO 2000068396 A2 20001116 (200066)\* EN 114[20]
  - RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL OA PT SD SE SL SZ TZ UG ZW
    - W: AE AG AL AM AT AU AZ BA BB BG BR BY CA CH CN CR CU CZ DE DK DM DZ EE ES FI GB GD GE GH GM HR HU ID IL IN 15 JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG UZ VN YU ZA ZW
    - AU 2000051327 A 20001121 (200112) EN
    - EP 1179075 A2 20020213 (200219) EN
      - R: AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT RO SE SI
- JP 2002543791 W 20021224 (200313) JA 156
- IN BENTZIEN J M
- L49 ANSWER 24 OF 87 SCISEARCH COPYRIGHT (c) 2008 The Thomson Corporation on STN
- TI Purification and partial characterization of a basic xylanase produced by thermoalkaliphlic Bacillus sp strain TAR-1
- SO BIOSCIENCE BIOTECHNOLOGY AND BIOCHEMISTRY, (APR 2000) Vol. 64, No. 4, pp. 887-890.
- ISSN: 0916-8451.
- AU Takahashi H; Nakai R; Nakamura S (Reprint)
- AN 2000:340808 SCISEARCH
- L49 ANSWER 25 OF 87 SCISEARCH COPYRIGHT (c) 2008 The Thomson Corporation on STN DUPLICATE 9
- TI Enhanced production, purification and characterisation of a novel cellulase-poor thermostable, alkalitolerant
  - xylanase from Bacillus circulans AB 16
- SO PROCESS BIOCHEMISTRY, (MAR 2000) Vol. 35, No. 8, pp. 849-856.
- ISSN: 0032-9592.
- AU Dhillon A; Gupta J K; Khanna S (Reprint)
- AN 2000:266722 SCISEARCH
- L49 ANSWER 26 OF 87 SCISEARCH COPYRIGHT (c) 2008 The Thomson Corporation on STN
- TI Production and characterization of thermostable xylanase
- and pectinase from Streptomyces sp QG-11-3
- SO JOURNAL OF INDUSTRIAL MICROBIOLOGY & BIOTECHNOLOGY, (JUN 2000) Vol. 24, No. 6, pp. 396-402.
  ISSN: 1367-5435.
- AU Beg Q K (Reprint); Bhushan B; Kapoor M; Hoondal G S
- AN 2000:616369 SCISEARCH
- L49 ANSWER 27 OF 87 SCISEARCH COPYRIGHT (c) 2008 The Thomson Corporation on STN DUPLICATE 10
- TI Isolation, purification and characterization of xylanase from
- Staphylococcus sp. SG-13 and its application in biobleaching of kraft pulp SO JOURNAL OF APPLIED MICROBIOLOGY, (FEB 2000) Vol. 88, No. 2, pp. 325-334.
- SO JOURNAL OF APPLIED MICROBIOLOGY, (FEB 2000) Vol. 88, No. 2, pp. 325-334. ISSN: 1364-5072.
- AU Gupta S; Bhushan B; Hoondal G S (Reprint)
- AN 2000:224873 SCISEARCH
- L49 ANSWER 28 OF 87 SCISEARCH COPYRIGHT (c) 2008 The Thomson Corporation on STN DUPLICATE 11
  - I Production of a thermostable alkali-tolerant
- xylanase from Bacillus circulans AB 16 grown on wheat straw WORLD JOURNAL OF MICROBIOLOGY & BIOTECHNOLOGY, (JUN 2000) Vol. 16, No. 4, pp. 325-327.

- ISSN: 0959-3993.
- AU Dhillon A; Khanna S (Reprint)
- AN 2000:698100 SCISEARCH
- L49 ANSWER 29 OF 87 SCISEARCH COPYRIGHT (c) 2008 The Thomson Corporation on STN DUPLICATE 12
- TI A cellulase-poor, thermostable, alkalitolerant xylanase produced by Bacillus circulans AB 16 grown on rice straw and its application in biobleaching of eucalyptus pulp
- SO BIORESOURCE TECHNOLOGY, (JUL 2000) Vol. 73, No. 3, pp. 273-277. ISSN: 0960-8524.
- AU Dhillon A; Gupta J K; Jauhari B M; Khanna S (Reprint)
- AN 2000:287403 SCISEARCH
- AN ZUUU: 28/403 SCISEARCH
- L49 ANSWER 30 OF 87 HCAPLUS COPYRIGHT 2008 ACS on STN
- TI A new record of a bacterial alkaline thermostable xylanase from an Egyptian soil
- SO Egyptian Journal of Biotechnology (2000), 7, 193-205
- CODEN: EJBIF7; ISSN: 1110-6093 AU Shabeb, M. S. A.
- AN 2000:194949 HCAPLUS
- DN 133:2286
- L49 ANSWER 31 OF 87 SCISEARCH COPYRIGHT (c) 2008 The Thomson Corporation on
- TI Xylanase activity and thermostratification during the thermogenic phase of industrial composting in aerated trenches
- SO WASTE MANAGEMENT & RESEARCH, (APR 2000) Vol. 18, No. 2, pp. 174-183. ISSN: 0734-242X.
- AU Lyon P F; Beffa T (Reprint); Fischer J L; Aragno M
- AN 2000:271416 SCISEARCH
- L49 ANSWER 32 OF 87 MEDLINE on STN DUPLICATE 13
- TI Homology model of a novel xylanase: molecular basis for high-
- thermostability and alkaline stability.
- 50 Journal of biomolecular structure & dynamics, (2000 Aug) Vol. 18, No. 1, pp. 137-44.
- Journal code: 8404176. ISSN: 0739-1102.
- AU Mande S S; Gupta N; Ghosh A; Mande S C AN 2000465734 MEDLINE
- L49 ANSWER 33 OF 87 SCISEARCH COPYRIGHT (c) 2008 The Thomson Corporation on STN DUPLICATE 14
- TI Immobilization of alkaliphilic Bacillus sp cells for
- xylanase production using batch and continuous culture
- SO APPLIED BIOCHEMISTRY AND BIOTECHNOLOGY, (MAY 2000) Vol. 87, No. 2, pp. 95-101.
  ISSN: 0273-2289.
- AU Mamo G; Gessesse A (Reprint)
- AN 2000:607893 SCISEARCH
- L49 ANSWER 34 OF 87 SCISEARCH COPYRIGHT (c) 2008 The Thomson Corporation on STN
- TI Electroelution as a simple and fast protein purification method: isolation of an extracellular xylanase from Bacillus sp CCMI 966
- SO ENZYME AND MICROBIAL TECHNOLOGY, (JUL 2000) Vol. 27, No. 1-2, pp. 95-99. ISSN: 0141-0229.
- AU Sa-Pereira P (Reprint); Duarte J; Costa-Ferreira M
- AN 2000:486930 SCISEARCH
- L49 ANSWER 35 OF 87 MEDLINE on STN DUPLICATE 15
- TI Overproduction and characterization of seleno-methionine xylanase T-6.

- SO Journal of biotechnology, (2000 Feb 28) Vol. 78, No. 1, pp. 83-6.
- Journal code: 8411927. ISSN: 0168-1656.
- Mechaly A; Teplitsky A; Belakhov V; Baasov T; Shoham G; Shoham Y AII
- AN 2000167558 MEDLINE
- L49 ANSWER 36 OF 87 MEDLINE on STN DUPLICATE 16
  - Extracellular xylanase production by two thermophilic
  - alkali-tolerant Bacillus strains in batch and continuous cultures.
- SO Zeitschrift fur Naturforschung, C. Journal of biosciences, (2000 Jan-Feb) Vol. 55, No. 1-2, pp. 66-9. Journal code: 8912155, ISSN: 0341-0382.
- ΔII Emanuilova E I; Dimitrov P L; Mandeva R D; Kambourova M S; Engibarov S A
- AN 2000201709 MEDLINE
- L49 ANSWER 37 OF 87 MEDLINE on STN DUPLICATE 17
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endo-1,4-beta-D-xylanase gene cloning and recombinant enzyme characterization (conference abstract)

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AU Edelmann K K H: Timonen M P L

AN 1997-12975 BIOTECHDS

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ΤI Purification and characterization of two xylanases from alkalophilic Cephalosporium sp. strain RYM-202;

thermostable alkaline endo-1,4-beta-D-

xylanase production

Appl.Environ.Microbiol.; (1996) 62, 9, 3480-82 ISSN: 0099-2240

CODEN: AEMIDF

Kang M K; Maeng P J; \*Rhee Y H AU

1996-13703 BIOTECHDS AN

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thermostable alkaline endo-1,4-beta-Dxvlanase isolation (conference abstract)

SO Abstr.Gen.Meet.Am.Soc.Microbiol.; (1996) 96 Meet., 566 CODEN: 0005P ISSN: 0067-2777

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DN 126:28341

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- DN 126:20298
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- ISSN: 0032-9592.
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pre-bleaching aid

50 International Symposium on Wood and Pulping Chemistry, 8th, Helsinki, June 6-9, 1995 (1995), Volume 2, 397-402 Publisher: Gummerus Kirjapaino Oy, Jyvaskyla, Finland.

CODEN: 65KDAY

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applications of molecular genetics to pulp bleaching

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- SO JOURNAL OF MOLECULAR CATALYSIS B-ENZYMATIC, (4 DEC 1995) Vol. 1, No. 1, pp. 7-15.
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- flexuosa for use in delignification and/or bleaching of pulp SO (1995) . US Patent 5437992; US Cl. 435/200 435/252.1 435/278 435/822
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- AN 97:5176 LIFESCI
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- TI Thermostable alkaline endo-1,4-beta-Dxylanase production;
- from Bacillus sp., for use in pulping and the paper industry
- AN 1994-07122 BIOTECHDS
- PI JP 06062839 8 Mar 1994
- L49 ANSWER 74 OF 87 BIOTECHDS COPYRIGHT 2008 THE THOMSON CORP. on STN
- TI New xvlanase;
  - Bacillus sp. thermostable and alkalistable native

or recombinant endo-1,4-beta-D-xylanase production and purification for use in the food, feedstuff and pulp industries

- AN 1994-05939 BIOTECHDS
- PI WO 9404664 3 Mar 1994
- L49 ANSWER 75 OF 87 BIOTECHDS COPYRIGHT 2008 THE THOMSON CORP. on STN
  - New Bacillus sp. AC13 with novel enzyme composition;

thermostable alkaline protease, endo-1,4-beta-D-xylanase and cellulase production for use in surfactant

composition, lignocellulose pulp treatment, etc.

- AN 1994-03564 BIOTECHDS
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- alkaliphilic strains of Bacillus spp. SO
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- TI Thermotoga maritima, Thermotoga neopolitana and Thermotoga thermarum recombinant thermostable endo-1,4-beta-D-xylanase production and characterization;
- application in delignification and bleaching AN
- 1993-14743 BIOTECHDS PΙ WO 9319171 30 Sep 1993
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- CODEN: BILED3; ISSN: 0141-5492
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- DN 118:54852
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  - alkalophilic, thermostable endo-1,4-beta-D-xylanase

isolation, of potential use in the pulp and paper industry (conference

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- CODEN: PBITE3
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- Comparison of thermostable xylanases having optimal
- activities at acidic, neutral, and alkaline pH values; recombinant endo-1,4-beta-D-xylanase preparation by

thermophilic bacterium gene expression in Thermoanaerobacter ethanolicus; potential enhanced ethanol production (conference abstract)

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- AII De Blois S; Wiegel J
- ΔN 1992-09381 BIOTECHDS
- L49 ANSWER 84 OF 87 BIOTECHDS COPYRIGHT 2008 THE THOMSON CORP. on STN
- ΤI Extremely thermophilic cellulose and hemicellulose degrading bacteria including isolates of the genus Dictyoglomus;

thermostable endo-1,4-beta-D-xvlanase

characterization from Thermoanaerobium sp., Clostridium thermohydrosulfuricum and Dictyoglomus thermophilum (conference abstract)

- Thermophiles Sci. Technol.; (1992) 54 SO
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- SO Jpn. Kokai Tokkvo Koho, 9 pp.
- CODEN: JKXXAF
- TN Imanaka, Tadayuki; Nishiya, Yoshiaki; Sogabe, Yukihiro
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PI JP 03061489 A		8 JP 1989-194307	19890728

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- thermoalkalophilus grown on agricultural wastes;

thermostable endo-1,4-beta-D-xylanase preparation on rice husk or bagasse culture medium; lignocellulose degradation

- Appl.Microbiol.Biotechnol.; (1990) 34, 1, 141-44 SO
- CODEN: EJABDD
- Rajaram S; \*Varma A AU 1991-04701 BIOTECHDS AN
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- TI Novel thermostable endo-1.4-beta-D-xylanase
- purification from Bacillus sp. culture
- preparation; AN 1990-05323 BIOTECHDS
- PΤ JP 01309684 14 Dec 1989

- L49 ANSWER 1 OF 87 SCISEARCH COPYRIGHT (c) 2008 The Thomson Corporation on STN DUPLICATE 1
- AB The number of possible applications of microbial xylanases in the pulp and paper industry is gradually increasing and several are approaching commercial use. However, the properties of commercial xylanases make them unsuitable for the real process of pulp bleaching. This industry needs xylanases which are free of cellulase, function for a long time, at least 3 h, at temperatures over 70 degreesC and which are active at a pH higher than 8.0. Newly isolated microbes, which produce cellulase-free xylanases and are easily grown using a low-cost agricultural waste substrate were investigated. The properties of cellulase-free xylanases from Streptomyces sp. Ab106 produced from a simplified low-cost substrate of cane bagasse matched the following industrial requirements: active and stable at temperatures of 50-80 degreesC, active at alkaline pH (PH 7-9), half-life at 70 degreesC, pH 9.0 of 5 h. (C) 2003 Elsevier Science Ltd. All rights reserved.
- L49 ANSWER 2 OF 87 SCISEARCH COPYRIGHT (c) 2008 The Thomson Corporation on STN
- AB In this study, the agricultural waste was used to screen for an organism that is capable of producing enzymes for degrading xylan and cellulose. Results showed that Streptomyces actuosus A-151, isolated from northern Taiwan, produced beta-xylanase when rice bran was used as the sole carbon source. Four xylanases, designated as FI-A, FI-B, FII-A, FII-B, were identified and purified from the culture filtrate of S. actuosus A-151. Their specific activities after purification were 41.3, 86.2, 20.4, 85.2 U/mg, respectively. The pH stability of the four enzymes was: FI-A, 5-8; FI-B, 3-8; FII-A, 5-9; and FII-B, 3-9. The optimum pH for FII-B was 4, and the others were near 5-6. The optimum temperatures for enzyme activities were 60degreesC for FII-B, and 70degreesC for the others. The thermal stability for all four enzymes were up to 60degreesC. The molecular weights of FI-A, FI-B, FII-A, and FII-B xylanases were 30,000, 45,000, 26,000, and 20,000, respectively, by sodium dodecylsulfate-polyacrylamide gel electrophoresis and 30,000, 43,000, 25,000, and 21,000, respectively, by gel filtration. Addition of xylan, shrimp and crab shell powder, and orange peel to the culture medium was found to enhance the production of xylanase. (C) 2003 Elsevier Inc. All rights reserved.
- L49 ANSMER 3 OF 87 BIOSIS COPYRIGHT (c) 2008 The Thomson Corporation on STN AB Enzymes such as family 11 xylanases are increasingly being used for industrial applications. Here, the cloning, structure determination and temperature-stability data of a family 11 xylanase, Xyn11X, from the alkali-tolerant Bacillus subtilis subspecies B230 are reported. This enzyme, which degrades xylan polymers, is being produced on an industrial scale for use in the paper-bleaching industry. Xyn11X adopts the canonical family 11 xylanase fold. It has a greater abundance of side chain to side chain hydrogen bonds compared with all other family 11 xylanase crystal structures. Means by which the thermostability of Xyn11X might be improved are suggested.
- L49 ANSWER 4 OF 87 SCISEARCH COPYRIGHT (c) 2008 The Thomson Corporation on STN
- AB The alkalophilic Bacillus circulans Dl was isolated from decayed wood. It produced high levels of extracellular cellulase-free xylanase. The enzyme was thermally stable up to 60degreesC, with an optimual hydrolysis temperature of 70degreesC. It was stable over a wide pH range (5.5-10.5), with an optimum pH at 5.5 and 80% of its activity at pH 9.0. This cellulase-free xylanase preparation was used to biobleach kraft pulp. Enzymatic treatment of kraft pulp decreased chlorine dioxide use by 23 and 37% to obtain the same kappa number (kappa number) and brightness,

respectively. Separation on Sephadex G-50 isolated three fractions with xylanase activity with distinct molecular weights.

- L49 ANSWER 5 OF 87 HCAPLUS COPYRIGHT 2008 ACS on STN
- AB Streptomyces Ab106 isolated from soil in northern Thailand produced xylanase of high activity at 60° at pH 9.0. A practical level of the xylanase production over 10 IU/mL was made with cane bagase medium, optimized by the mixture design method. Pulp breaching was tested using this preparation at 60° at pH 9.0, resulting in a same level of breaching as that with chlorine. It is better than xylanase from Bacillus with respect to available pH and stability, currently being tested for an industry.
- L49 ANSWER 6 OF 87 HCAPLUS COPYRIGHT 2008 ACS on STN
- AB A review. Xylanase is an important enzyme and has various applications in industry. Its applications in pulp and paper industry are especially attractive

since it can reduce some environmental pollutants. Its higher thermostability and optimal activity at alkaline pH are of particular importance to the paper and pulp industry due to the demands of the enzymic reactions conditions. There are two ways to obtain such enzymes, one is to screen high-productivity microorganism from extreme environment, the other is to make use of site-directed mutagenesis as well as other high technol. alter wild enzyme more desirable. The advances in xylanase on the two aspects were reviewed.

- L49 ANSWER 7 OF 87 SCISEARCH COPYRIGHT (c) 2008 The Thomson Corporation on STN DUPLICATE 2
- AB Overproduction of cellulolytic enzymes through conventional nuclear transformation approaches posed a major challenge as they can potentially degrade the cell wall components and thereby affect transgenic plant growth and development. In this study, we have tested the possibility to over produce an alkali-thermostable xylanase gene from Bacillus sp. Strain NG-27 in tobacco plants through chloroplast expression. Our results showed that the xylanase expression can reach up to 6% of the total soluble protein, a value comparable to high level expression reported for several non-cellulolytic proteins in tobacco chloroplasts. The chloroplast-expressed xylanase retained its activity even when the leaves were dried under sun or at 42degreesC, offering flexibility in the agricultural system in transport and storage. The recombinant enzyme was purified to homogeneity using single step chromatography with more than 85% recovery. Most importantly, transgenic plants were indistinguishable from the control untransformed plants in their morphology, growth and in seed setting. These results open up new avenues for large scale production of several other industrially useful cellulolytic enzymes through chloroplast expression.
- L49 ANSWER 8 OF 87 BIOTECHDS COPYRIGHT 2008 THE THOMSON CORP. on STN AB DERWENT ABSTRACT:

NOVELTY - A chemical pulp is bleached by exposing pulp to an acidic bleaching stage to produce a partially bleached pulp and treating with a thermophilic, alkalophilic xylanase in an alkaline extraction stage with a final DH of 8-14.

BIOTECHNOLOGY - Preferred Enzyme: The thermophilic, alkalophilic xylanase comprises a genetically modified xylanase, comprising a family 11 xylanase from Trichoderma. The xylanase is a genetically modified Trichoderma reesei, selected from Trx HML 75A, 105H, 125A, 129E, 132R, 135R, 144R, 157D, 161R, 162H, 165H; 165H; TxxHML 75A, 105H, 125A, 129E, and TrxHML 75A, 105H, 125A, 129E, and TrxHML 75A, 105H, 125A, 129E, ind TrxHML 75A, 105H, 125A, 129E, 137E, 144R, 157D, 161R, 162H, 165H (each sequence having 190 amino acids given in the specification), where HML denotes the mutations 10H, 27M and 29L. The

xylanase comprises BioBrite xylanase or a wild type xylanase. Preferred Method: The alkaline extraction is performed at 60-120degreesC at a final pH of 9-11.5 for 30-120 minutes. The alkaline extraction is performed using oxygen and/or hydrogen peroxide. 0.1-10 kg of oxygen and hydrogen peroxide is present per ton of pulp. The partially bleached pulp is treated with a second xylanase at pH 8-14. The second xylanase is identical to the first xylanase. The pulp is treated with the first xylanase after alkaline oxygen delignification stage. The enzymatic treatment is performed in condition different from the alkaline extraction stage. Alternately, the chemical pulp is exposed to a chemical bleaching stage to produce a partially bleached pulp. The partially bleached pulp is incubated with an extraction filtrate containing the xylanase and subsequently washed with water to produce a papricycle washed xylanase treated pulp. The papricycle pulp is treated with the xylanase at a final pH of 8-14. Then the extraction filtrate is removed from the extract.

USE - For bleaching pulp using xylanase.

ADVANTAGE - The method enables to ensure proper mixing of the enzyme with pulp, to control and monitor process conditions such as pH, temperature, enzyme dosage and incubation time. The method does not necessarily require significant changes to existing pulp bleaching equipment, such as purchasing and implementing costly vessels for performing xylanase treatment. By carrying out xylanase treatment in an alkaline extraction stage, little or no acid is required to adjust the pH of the pulp prior to xylanase addition. The reduction or elimination of acid reduces corrosion of mill equipment and the costs associated with a pulp bleaching process. The addition of xylanase after an acidic bleaching stage, or before and after a bleaching stage increases the overall effect of enzyme treatment. The pulp bleaching method also reduce the amount of chemicals required to bleach pulp and also reduce the amount of chemicals required to bleach pulp and also reduce the amount of chlorinated effluent waste produced by a pulp bleaching process.

EXAMPLE - Unbleached hardwood kraft pulp was incubated at 60 degrees C, at initial pH 9.4 for 60 minutes to simulate the conditions of an enzyme treatment stage. The pulp was washed with water. 15 g of sample of pulp was subjected to chlorine dioxide bleaching stage. Chlorine dioxide was added to the pulp and the system was maintained in a heat-sealable plastic bag. The pulp mixture was cooled to 4 degrees C to minimize evaporation. The kappa factor was recommended to be about 0.17 to avoid formation of furans and dioxins. The pulp was adjusted to a 10% consistency with tap water and the initial pH was adjusted to 9.4 with sodium hydroxide. The pulp was heated to 60degreesC and a genetically modified Trichoderma reesei xylanase (having Trx HML 75A, 105H, 125A, 129E, 132R, 135R, 144R, 157D, 161R, 162H, 165H at amount of 2.0 units/g of pulp with the enzyme stock at 33 units/ml was added to the pulp. Pulp was treated in a similar manner but with a thermophilic, alkalophilic, xylanase in the alkaline extraction stage and exhibited a kappa number of 4.8. (61 pages)

L49 ANSWER 9 OF 87 BIOTECHDS COPYRIGHT 2008 THE THOMSON CORP. on STN AB DERWENT ABSTRACT:

NOVELTY - A non-naturally occurring xylanase activity (XA) protein (I) comprising an amino acid sequence less than 97% identical to a naturally occurring Bacillus circulans xylanase, where the protein has been modified to exhibit enhanced thermophilicity, alkalophilicity, or thermostability relative to naturally occurring B.circulans xylanase, and has at least 5 amino acid substitutions, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for: (1) a recombinant nucleic acid (II) encoding (I); (2) an expression vector (III) comprising (II); (3) a host cell (IV) comprising (II) or (III); (4) producing (I); (5) a non-naturally occurring XA conformer (V) having a

three dimensional backbone structure that substantially corresponds to the three dimensional backbone structure of B.circulans xylanase, where the amino acid sequence of the conformer and the amino acid sequence of the B.circulans xylanase are less than about 97% identical; (6) a bleaching agent (VI) comprising (I) as an active ingredient; (7) a non-naturally occurring XA amino acid sequence selected from a group as given in the specification; (8) a non-naturally occurring XA nucleic acid sequence selected from a group as given in the specification; and (9) a XA library comprising variants of XA, where the variants have been altered to exhibit enhanced alkalophilicity, thermophilicity, or thermostability relative to a naturally occurring B.circulans xylanase.

WIDER DISCLOSURE - Variants of (I) are also disclosed.

BIOTECHNOLOGY - Preparation: (I) is prepared by culturing (IV) under conditions suitable for the expression of (II) and recovering the expressed XA protein. Preferred Protein: (I) comprises at least three among the substitutions at positions chosen from 5, 7, 11, 26, 28, 30, 37, 39, 53, 58, 63, 64, 65, 67, 79, 80, 83, 84, 85, 88, 96, 98, 100, 102, 103, 105, 109, 110, 115, 118, 125, 128, 129, 130, 132, 136, 142, 144, 147, 148, 149, 150, 152, 156, 158, 160, 167, 168, 171, 176, 180, and 182. In (V), at least 90% or 100% of the non-identical amino acids are in the core region of the conformer.

USE - A bleaching agent comprising (1) is useful for bleaching pulp, by contacting pulp with the bleaching agent, which further comprises chemical bleaching and/or an alkali extraction before, after or during contacting pulp with the bleaching agent (claimed). XA proteins and nucleic acids are useful in the bicconversion of lignocellulosic materials to fuels, for clarifying juice and wine, extracting coffee, plant oils and starch, producing food thickeners, altering texture in bakery products, e.g., improving the quality of dough, help bread rise and processing of wheat and corn for starch production, use as animal food additives to aid in the digestibility of feedstuffs and in the washing of super precision devices and seniconductors.

ADVANTAGE - (I) is more alkalophilic, thermophilic and thermostable and hydrolyzes a substrate more efficiently than B.circulans xylanase.

EXAMPLE - Sequences for novel thermostable, thermophilic and/or alkaliphilic xylanase activity (XA) proteins were designed by optimizing residues in the core of the protein, residues around D83, residues around the helix region, and residues around the active site region using Protein Design Automation (PDA) as described in W098/47089. By visual inspection, the following residues were identified as belonging to the Core of Bacillus circulans xylanase: Y26, V28, W30, F36, V38, I51, Y53, A55, W58, G62, G64, L66, L68, G70, T72, I77, Y79, V81, Y105, I107, S130, A142, I144, F146, W153, M169, T171, G173, S176, G178, S180, V182, and V184. A rotamer group was assigned to each CORE position which allowed this position to become any phobic residue with the exception of methionine (i.e. Ala, Val, Leu, Ile, Phe, Tyr, and Trp) plus the original wild type residue. In the following PDA design only the CORE residues were allowed to mutate to any amino acid rotamer restricted by the definition of the rotamer group assigned to this position. The rest of the protein was treated as a template, with fixed coordinates. An energy cutoff of 50 kcal/mol for the rotamer/template energy was used to exclude unfavorable rotamers. The Van der Waals radius was scaled by a factor of 0.9 and the solvation model 2 as defined by Street and Mayo was used. The ground state rotamer sequence was extracted from all the possible rotamer sequences using the Dead End Elimination (DEE) method. To check for other low energy sequences a Monte Carlo (MC) search was performed starting from the DEE ground state. The PDA calculation resulted in a ground state sequence given in the specification. This sequence showed 13 mutations when compared to the wild type B.circulans xylanase sequence, Y26F, V28I, W30F, Y53F, W58F,

G64V, Y79F, Y105F, A142L, T171L, S176A, S180A, and V182I. This state showed 93% identity with the complete wild type B.circulans xylanase sequence and had 60% identity in the designed positions with the wild type sequence. Using the Monte Carlo technique a list of low energy sequences was generated. Out of the lowest 1000 sequences generated by Monte Carlo none had more than 20 mutations from the wild type sequence and out of the lowest 101 sequences none had more than 18 mutations. Any protein sequence showing mutations at the above positions generated a more stable and/or active XA protein. (121 pages)

- L49 ANSWER 10 OF 87 MEDLINE on STN DUPLICATE 3
- AB Cellulase-free xylanase was produced by Streptomyces sp. Abl06 using came bagases as the substrate at 55 degrees C. Its maximum activity was 13 IU without cellulase and mannanase activities. Its profiles were investigated. Its optimum temperature and pH were 60 degrees C and 6.0, respectively. More than 70% of its activity was remained at 60 degrees C at pH 9. This enzyme was quite stable and exhibited an active of more than 70% for 144 h at 60 degrees C, and of more than 80% for 144 h at 60 degrees C, and of more than 80% for 144 h at 40 degrees C, pH 9. This thermo-tolerant and alkaline-tolerant xylanase can be used in the pulp bleaching process.
- L49 ANSMER 11 OF 87 SCISEARCH COPYRIGHT (c) 2008 The Thomson Corporation on STN
  AB The xylanase A (XynA) from the alkaliphilic
  - The xylanase A (XynA) from the alkaliphilic Bacillus halodurans C-125 and the xylanase B (XynB) from Clostridium stercorarium F9 were subdivided into four fragments at highly homologous regions present in their primary structures: an amino-terminal region (A or a), a region containing the putative proton donor (P or p), a region containing the putative catalytic nucleophile (N or n), and a carboxyl-terminal region (C or c). Six chimeric xylanases were constructed by the selective substitution of the four fragments using an overlapping PCR technique. Two of the six xylanases, APnc and Apnc (regions originating from XynA are denoted by upper case letters and those from XynB are denoted by lower case letters), were produced in Escherichia coli while the other four xylanases were obtained only as inclusion bodies. The APnc and Apnc chimeric enzymes were purified by column chromatography using Ni-NTA agarose and DEAE-Toyopearl. The respective pH and temperature stabilities of the purified enzymes were observed from pH 5.6 to 11.6 and up to 45degreesC for APnc, and from pH 5.6 to 11.2 and up to 45degreesC for Apnc. Thus, these enzymes were slightly less stable than the parental xylanases. An assessment of the pH-activity relationships for the chimeric xylanases employed p-nitrophenyl-beta-Dxylobioside as the substrate in determinations of the k(cat) values. The pK(a1) values for the APnc and Apnc chimeric enzymes were 4.3 and 4.2, respectively, which were almost identical to those for the parental xylanases. In contrast, the pK(a2) values obtained for APnc and Apnc were 9.1 and 8.5, respectively; these values fall between those for the parental xylanases, XynA (9.4) and XynB (7.8). These results indicate that the main regions necessary to maintain the high pK(a2) value of XvnA locate in the A and P sections.
- L49 ANSWER 12 OF 87 SCISEARCH COPYRIGHT (c) 2008 The Thomson Corporation on STN
- AB acillus sp. CCMI 966, characterised as Bacillus subtilis, has a duplication time of about 24 min. It produces at least two extracellular xylanases, Xyl I and Xyl II. The extracellular xylanase activity seems to be strongly correlated with the biomass growth profile. The Xyl II isoenzyme was purified by ammonium sulphate precipitation and anionic exchange chromatography, with a purification factor of 8.3. The molecular weight of the isoenzyme was estimated by SDS-PAGE revealing that Xyl II is a multimeric enzyme with a catalytic subunit of about 20 kDa. Under non-denaturing conditions, a molecular weight of about 340 kDa was

obtained by native PAGE gel and of 20 kDa by gel filtration chromatography. The enzyme showed an optimum pH and temperature of 6.0 at 60 degreesC. Xyl II was stable at 40 degreesC for 180 min at pH 6.0. The specificity of Xyl II for different substrates was evaluated. Xyl II presents a higher affinity towards OSX. with a K-m of 1.56 g 1(-1) and showed the ability to hydrolyse laminarin, with a K-m of 1.02 g 1(-1). Xylotetraose is the main product of xylan degradation. The Xyl II ability for binding to cellulose and/or xylan was also studied. (C) 2002 Elsevier Science B.V. All rights reserved.

- L49 ANSWER 13 OF 87 SCISEARCH COPYRIGHT (c) 2008 The Thomson Corporation on DUPLICATE 4
- AB The characterization of a partially purified, cellulase-free, thermostable alkaline xylanase from thermoalkalophilic Bacillus sp. JB-99 was investigated. The xylanase production was the highest when birchwood xylan was added to a medium containing finely powdered rice bran, showing 4,826 IU ml(-1) of activity for 15 h of incubation. The partially purified xylanase exhibited an optimum temperature and pH at 70degreesC and 10, respectively. The enzyme was stable at pH 5-11 at 50degreesC. The xylanase activity was strongly inhibited by Hg2+, while dithiothreitol, cysteine, and beta-mercaptoethanol enhanced the activity.
- L49 ANSWER 14 OF 87

MEDLINE on STN We studied the effects of increase in the number of surface arginines on the enzyme activity and stability of Trichoderma reesei endo-1,4-beta-xylanase II. The number of arginines was increased in two mutant series. The first set contained six arginines on different sides of the protein surface. These arginines had no significant effect on the thermostability. However, the optimal pH region became narrower. Another series of five arginines was engineered into the 'Ser/Thr surface', formed of part of the double-layered beta-sheet located on one side of the 'right-hand-like' xylanase. These mutations shifted the activity profile to the alkaline region by approximately 0.5-1.0 pH units. In addition, the arginines on the Ser/Thr surface increased the enzyme activity at high temperature, although the enzyme stability in the absence of substrate decreased significantly at 50-55 degrees C. In the presence of the substrate, the thermostability increased 4-5-fold at 60-65 degrees C. Thus, the substrate neutralized the destabilizing effect of Ser/Thr surface arginines and revealed a stabilizing effect of the same mutations. The stabilizing effect of arginines at high temperatures was seen clearly only when five arginines were introduced into the Ser/Thr surface.

L49 ANSWER 15 OF 87 MEDLINE on STN DUPLICATE 6 AB Eighty-two amino acid sequences of the catalytic domains of mature endoxylanases belonging to family 11 have been aligned using the programs MATCHBOX and CLUSTAL. The sequences range in length from 175 to 233 residues. The two glutamates acting as catalytic residues are conserved in all sequences. A very good correlation is found between the presence (at position 100) of an asparagine in the so-called 'alkaline' xylanases, or an aspartic acid in those with a more acidic pH optimum. Four boxes defining segments of highest similarity were detected; they correspond to regions of defined secondary structure: B5, B6, B8 and the carboxyl end of the alpha helix, respectively. Cysteine residues are not common in these sequences (0.7% of all residues), and disulfide bridges are not important in explaining the stability of several thermophilic xylanases. The alignment allows the classification of the enzymes in groups according to sequence similarity. Fungal and bacterial enzymes were found to form mostly separate clusters of higher similarity.

STN DUPLICATE 7

- AB Cellulase-free xylanase was produced by Streptomyces sp. Abl06 from the agricultural waste cane bagasse. The effect of external factors pH and temperature on the xylanase production was studied and the optimization by using the central composite experimental design was investigated. The effects of pH and temperature were significant for the xylanase production. A second-order quadratic model and a response surface method showed that the optimum condition for xylanase production was 50degreesC and pH 7. The maximum yield of xylanase was about 15 IU without cellulase and manannase activities. (C) 2002 Elsevier Science B.V. All rights reserved.
- L49 ANSWER 17 OF 87 HCAPLUS COPYRIGHT 2008 ACS on STN
- AB Since the discovery by Viikari et al. (1986) that xylanases can be used to enhance kraft pulp bleaching, there has been strong interest in thermostable and alkaliphilic xylanases. Here we present a computational approach to design such an enzyme rationally. Though it is impossible to screen the complete sequence space of a protein by purely exptl. methods alone, Xencor's proprietary Protein Design Automation (PDATM) technol. offers a way to screen complete proteins computationally. We show how PDATM can be used as a fast computational prescreen that reduces the complexity of the sequence space to a number accessible for exptl. screening techniques. The Bacillus circulans xylanase has been chosen as an example for this approach. Initial results of a small focused design towards improving the thermostability and alkaliphilicity will be presented.
- L49 ANSWER 18 OF 87 MEDLINE on STN DUPLICATE 8
  AB An alkalophilic Aspergillus nidulans KK-99 produced an alkaline, thermostable xylanase (40 IU/ml) in a basal medium supplemented with wheat bran (2% w/v) and KNO3 (at 0.15% N) pH 10.0 and 37 degrees C. The partially purified xylanase was optimally active at pH 8.0 and 55 degrees C. The xylanase was stable in a broad pH range of 4.0-9.5 for 1 h at 55 degrees C, retaining more than 80% of its activity. The enzyme exhibited greater binding affinity for xylan from hardwood than from softwood. The xylanase activity was stimulated (+25%) by Na+ and Fe2+ and was strongly inhibited (maximum by 70%) by Tween-20, 40, 60, SDS, acetic anhydride, phenylmethane sulphonyl fluoride, Triton-X-100. The xylanase dose of 1.0 IU/q dry weight pulp qave optimum bleach boosting of

Kraft pulp at pH 8.0 and temperature 55 degrees C for 3 h reaction time.

- L49 ANSWER 19 OF 87 HCAPLUS COPYRIGHT 2008 ACS on STN
- AB A recent trend in biobleaching of paper pulps is to apply xylanases which are active and stable at alkaline pH and elevated temps. For this purpose, thermophilic bacteria and fungi are particularly suitable as enzyme producers. Solid-state fermentation (SSF) of xylanase has been found advantageous over the classical submerged fermentation method of enzyme production as it can offer the possibility of a direct use of in-situ xylanase for pulp pretreatment and bleaching without a prior downstream processing of enzyme. The pulp, which is initially used as a carbon source for enzyme production, subsequently becomes the target substrate of enzyme application. This approach could certainly improve the economics and enhance the efficiency of the biobleaching technol, due to the operational simplicity of SSF and production of substrate-specific enzymes in a water-restricted environment. In this study, microbial production of xylanase by SSF was evaluated using soda-ag pulp from Eucalyptus grandis. Screening of a number of thermophilic and thermotolerant fungal isolates yielded xylanase activities of 1,000-6,000 U/g dry pulp containing very low cellulase (<0.1 FPU/g). The in-situ produced enzymes were then assessed for their potential to increase pulp bleachability in an ECF bleach sequence. A brightness gain over control of up to 1.5 points was attained depending on the amount of SSF enzyme

applied. It was shown that the use of in-situ xylanase in biobleaching of eucalypt soda-ag pulp could be as efficient as com. available enzyme products. Using the best-performing isolate from the bleaching trials, the optimum moisture content, pH and time course of fermentation process were determined Media optimization studies with Plackett Burman exptl. design to enhance xylanase production by SSF (over 9,000 U/q) were carried out. Potential advantages and benefits of in-situ SSF production and application of xylanase in the pulp and paper industry were discussed.

- L49 ANSWER 20 OF 87 HCAPLUS COPYRIGHT 2008 ACS on STN
- A thermostable and alkali-tolerant xylanase -producing Bacillus species was shown to be the most potent microorganism for bio-pulping and bio-bleaching. In solid-state conditions, moisture content is 75%, pH is 8.5, and temperature 50°. It required only 4 h. for maximum in situ solubilization of xylan. It gave a 60% decrease in kappa number of the treated pulp, which is at par with conventional pulping. Xylanase treatment of raw material and pulp with the Bacillus species saved nearly 60% of energy and 2 h time required for the whole process of kraft pulping.

=> d ab 21-40

- L49 ANSWER 21 OF 87 HCAPLUS COPYRIGHT 2008 ACS on STN A recombinant Bacillus expressing a gene for a thermostable, alkali-stable xylanase and its use for preparation of the xylanase is disclosed. The xylanase may be used in wood pulp delignification (no data). Thus, the gene for a thermostable, alkali-stable xylanase was cloned from Bacillus NG 27/MTCC B0013 and sequenced. This gene was ligated into E. coli-B. subtilis shuttle plasmid pRB373 and the resulting recombinant plasmid was transferred to xylanase-neg. B. subtilis to produce recombinant strain MTCC BPL0010. MTCC BPL0010 was cultured for 12-72 h in a shake flask. Optimal xylanase production was observed for 48-60 h at 37° and pH 7.0. This recombinant B. subtilis produced 200 times more enzyme than did the parent strain.
- L49 ANSWER 22 OF 87 SCISEARCH COPYRIGHT (c) 2008 The Thomson Corporation on
- AB The catalytic domain of a xylanase from the anaerobic fungus Neocallimastix patriciarum was made more alkalophilic through directed evolution using error-prone PCR. Transformants expressing the alkalophilic variant xylanases produced larger clear zones when overlaid with high pH, xylan-containing agar. Eight amino acid substitutions were identified in six selected mutant xylanases. Whereas the wild-type xylanase exhibited no activity at pH 8.5, the relative and specific activities of the six mutants were higher at pH 8.5 than at pH 6.0. Seven of the eight amino acid substitutions were assembled in one enzyme (xyn-CDBFV) by site-directed mutagenesis. Some or all of the seven mutations exerted positive and possibly synergistic effects on the alkalophilicity of the enzyme. The resulting composite mutant xylanase retained a greater proportion of its activity than did the wild type at pH above 7.0, maintaining 25% of its activity at pH 9.0, and its retention of activity at acid pH was no lower than that of the wild type. The composite xylanase (xyn-CDBFV) had a relatively high specific activity of 10 128 mu mol glucose.min(-1.)(mg protein)(-1) at pH 6.0. It was more thermostable at 60 degreesC and alkaline tolerant at pH 10.0 than the wild-type xylanase. These properties suggest that the composite mutant xylanase is a promising and suitable candidate for paper pulp bio-bleaching.

WO 2000068396 A2 UPAB: 20050412

AB

- NOVELTY Non naturally occurring XA protein (I) comprising an amino acid (aa) sequence less than 97% identical to a naturally occurring Bacillus
  - circulans xylanase, is modified to exhibit enhanced
  - thermophilicity, alkalophilicity or thermostability
    - relative to the naturally occurring B. circulans xylanase.
    - DETAILED DESCRIPTION INDEPENDENT CLAIMS are also included for the following:
    - (1) a non-naturally occurring XA conformer (II) with a three dimensional backbone structure that corresponds to the three dimensional backbone structure of B. circulans xylanase and has less than 97% sequence identity to the aa sequence of B. circulans xylanase;
      - (2) a recombinant nucleic acid (III) encoding (I);
      - (3) an expression vector (IV) comprising (III);
      - (4) a host cell comprising (III);
      - (5) a host cell comprising (IV);
    - (6) a method of producing (I) comprising culturing the host cell of
    - (4) under conditions suitable for expression of the nucleic acid;
      (7) a bleaching agent comprising a XA protein as an active
    - ingredient; and

      (8) a method for bleaching pulp comprising contacting pulp to be
    - bleached with the agent of (7).  ${\tt USE - (I) \ is \ used \ as \ the \ active \ compound \ in \ a \ bleaching \ agent \ which}$
- is used for bleaching pulp (claimed).
- L49 ANSWER 24 OF 87 SCISEARCH COPYRIGHT (c) 2008 The Thomson Corporation on STN
- AB A basic xylanase was purified from the culture supernatant of thermoalkaliphilic Bacillus sp. strain TAR-1. Its molecular mass and isoselectric point were 23 kDa and > pH 9.3, respectively. The enzyme showed a broad pH profile and was optimally active at 70 degrees C. Analyses of xylan-degradation products and N-terminal amino acid sequence revealed that the enzyme would be a family 11/G endoxylanase.
- L49 ANSWER 25 OF 87 SCISEARCH COPYRIGHT (c) 2008 The Thomson Corporation on STN DUPLICATE 9
- AB A thermophilic Bacillus circulans AB 16, isolated from a garbage dump produced novel extracellular cellulase-poor, thermostable xylanase in basal medium containing rice straw. The xylanase vield was enhanced more than two and a half fold in the presence of tryptone and other media modifications. The highest xylanase activity obtained in liquid culture was 55 IU/ml. Two xylanases Xyl A and Xyl B were purified to homogenity by Q Sepharose and Sepharose 6B chromatograph. Xyl A (M-r 30 000) had a pH optima of 6 and a temperature optima of 75-80 degrees C. while Xvl B (M-r 22 000) also had a pH optima of 6 but with a temperature optima of 65-70 degrees C. Both Xyl A and Xyl B had a pH optima of 6 but retained 46% activity at pH 8. Xyl A retained 70% activity at 65 degrees C, pH 9 and 2 h incubation while Xyl B retained 34% under the similar conditions. Both Xyl A and Xyl B showed 90% inhibition in activity by 1 mM Hq2+. The hydrolysis pattern of Xyl A yielded mainly xylobiose with lower amount of xylo-oligosaccharides and xylose while Xyl B vielded mainly higher oligosaccharides with lesser amounts of xylobiose and negligible amounts of xylose. The crude enzyme from B. circulans AB 16 showed higher chromophore release of 0.360 U as compared to Xyl A and Xyl B with a chromophore release of 0.115 and 0.069 units and thus would be more useful for pulp bleaching than purified xylanases. (C) 2000 Elsevier Science Ltd. All rights reserved.
- L49 ANSWER 26 OF 87 SCISEARCH COPYRIGHT (c) 2008 The Thomson Corporation on STN
- AB Streptomyces sp. QG-17-3, which produces a cellulase-free thermostable xylanase (96 IU ml(-1)) and a pectinase (46

IU ml(-1)), was isolated on Horikoshi medium supplemented with 1% w/v wheat bran. Carbon sources that favored xylanase production were rice bran (82 IU ml(-1)) and birch-wood xylan (81 IU ml(-1)); pectinase production was also stimulated by pectin and cotton seed cake (34 IU ml(-1)) partially purified xylanase and pectinase were optimally active at 60 degrees C. Both enzymes were 100% stable at 50 degrees C for more than 24 h. The half-lives of xylanase and pectinase at 70, 75 and 80 degrees C were 90, 75 and 9 min, and 90, 53 and 7 min, respectively. The optimum pH values for xylanase and pectinase were 8.6 and 3.0, respectively, at 60 degrees C. Xylanase and pectinase were stable over a broad pl-i range between 5.4 and 9.4 and 2.0 to 9.0, respectively retaining more than 85% of their activity. Ca2+ stimulated the activity of both enzymes up to 7%, whereas Cd2+, Co2+ CT3+, iodoacetic acid and iodoacetamide inhibited xylanase up to 35% and pectinase up to 63%; at 1 ml, Hg2+ inhibited both enzymes up completely.

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AB

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A haloalkalophilic Staphylococcus sp. SG-13 produced an alkalistable xylanase in wheat bran medium. A 12-fold purification was achieved by using standard purification techniques. The purified xylanase exhibited a dual pH optima of 7.5 and 9.2. The optimum temperature for enzyme activity was 50 degrees C. The enzyme was stable at 50 degrees C for more than 4 h. The xylanase exhibited K-m and V-max values of 4 mg ml(-1), 90 mu mol min(-1) per mg for birchwood xylan and 7 mg ml(-1), 55 mu mol min(-1) per mg for oatspelt xylan, respectively. The substrate binding affinity of xylanase was more for oatspelt xylan but birchwood xylan was hydrolysed more rapidly. The xylanase activity was stimulated by Fe2+, Ni2+, Cu2+ and dithiothreitol up to 60% and was strongly inhibited in the presence of Co2+, Hg2+, Pb2+, phenyl methane sulphonyl fluoride, ethylenediaminetetraacetic acid, and acetic anhydride up to 100%. The xylanase dose of 1.8 U g(-1) moisture free pulp, exhibited bleach boosting of kraft pulps optimally at pH 9.5-10.0 and 50 degrees C after 4 h of reaction time. Pretreatment of pulp with xylanase and its subsequent treatment with 8% hypochlorite, reduced the kappa number by 30%, enhanced the brightness and viscosity by 11% and 1.8%, respectively, and improved the paper properties such as tensile strength and burst factor up to 10% and 17%, respectively.

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Bacillus circulans AB 16 was able to produce 50 IU/ml of xylanase, with negligible cellulase activity when grown on untreated wheat straw. The pH optimum of the crude enzyme was 6-7 with a temperature optimum of 80 degrees C. The enzyme showed high pH and thermal stability retaining 100% activity at 60 degrees C, pH 8 and 9 after 2.5 h of incubation. The residual activity at 70 degrees C after 2.5 h was 62% and 45% at pH 8 and 9, respectively. At 75 degrees C only 22.2% activity remained at pH 8 after 1 h incubation. Since Kraft pulp is alkaline this enzyme could be used for prebleaching of pulp at temperatures up to 70 degrees C without pH adjustment.

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AB Bacillus circulans AB 16 isolated from a garbage dump produced appreciable quantities (19.28 IU/ml) of extracellular thermophilic xylanase, but negligible quantities of cellulase, when grown on 0.3% xylan. The optimum pH for the enzyme was 6.0-7.0, but it was stable over a wide range of pH (5.0-9.0). The optimum temperature was 80 degrees C. The organism produced 20.6 IU/ml of xylanase in shake flask on rice straw, an inexpensive lignocellulosic biomass. Glucose, fructose, xylose and other sugars induced enzyme levels only in the range 0.82-2.52 IU/ml.

The crude enzyme produced on rice straw showed good thermal and pH stability, retaining 67% activity after 1 h at 70 degrees C, pH 9 and 84.5% activity after 2 h at 65 degrees C, pH 9. The enzyme had a half-life of 24 h at 70 degrees C, pH 7. When the xylanase from B. circulans AB 16 was used in the prebleaching of eucalyptus Kraft pulp the amount of chlorine was reduced by 20% without any decrease in brightness. The viscosity of xylanase-treated pulp was 9.5-9.7 cp, whereas that of the pulp treated exclusively with chlorine was 9.2 cp. (C) 2000 Elsevier Science Ltd. All rights reserved.

- L49 ANSWER 30 OF 87 HCAPLUS COPYRIGHT 2008 ACS on STN
- Twenty-seven isolates were isolated as an alkaline xylanase -producing microorganisms from soil samples and hemicellulosic materials. Identification was carried out by comparison with reference strains cited in Bergey's Manual of Systematic Bacteriol. (1986). The present isolates were investigated for their xylanase activity in three stages using wheat bran extract agar medium with xylan. One of the isolates, identified as Bacillus caldotenax BS - 48 produced 118 times more xylanase activity than other isolates. Maximum enzyme produced was observed after 96 h of growth with pH range of 9-12 at 55°C. The crude enzyme was stable for 30 min at 55°C and for >2 h at pH 8-11. Thus, it is believed that the present xylanases may be considered as a new record alk . thermostable xylanase from an Egyptian soil.
- L49 ANSWER 31 OF 87 SCISEARCH COPYRIGHT (c) 2008 The Thomson Corporation on
- AB Extracellular xylanase activity and thermostratification were monitored during the thermogenic phase of industrial composting (mainly garden waste) in aerated trenches. Xylanase activity was assayed at different temperatures ansi pH by incubation of clarified compost extracts with xylan. The highest xylanase activity and temperatures were measured during the summer (when waste was rich in freshly cut grass). Xylanase activity was higher in the peripheral layers of the trenches, at moderately high temperatures (48 to 68 degrees C), than in the hottest central layers (70 to 78 degrees C). Optimal temperature for the xylanases was between 60 and 80 degrees C at pH6, depending on the temperature of the sampling site. The thermostability of the xylanases from surface samples was high until 60 degrees C, moderate at 70 degrees C and weak at 90 degrees C. Our results show a broad thermostratification of the compost mass. Frequent turnings of the compost stimulate xylan degradation by redistributing the substrates, the free enzymes and the microorganisms.
- L49 ANSWER 32 OF 87

AB

MEDLINE on STN DUPLICATE 13 Xylanases form enzymes of considerable interest to a variety of biotechnological industries. Their industrial usage is especially attractive since they can replace some of the environmental pollutants, and are economically viable. Those with higher thermostability and optimal activity at alkaline pH are of particular importance to the paper and pulp industry due to the demands of conditions under which the enzymatic reactions are carried out. We have earlier isolated a xylanase from Bacillus sp. NG-27, which is active both at high temperature as well as at alkaline pH. In order to find out factors responsible for the adaptation of this enzyme to the extreme conditions, three dimensional structure of NG-27 xylanase has now been obtained by homology modelling. The tertiary structure shows TIM barrel fold consisting of 8 parallel beta-strands surrounded by alpha-helices. The active site is located at the carboxy terminal end of the TIM barrel. Factors which contribute to the thermostability of the enzyme are increased number of salt bridges. The salt bridges occur remarkably on one face of alpha-helices, with oppositely charged residues occupying i, i+4, i+7 positions. A solvent shielded salt bridge interaction is also observed, which is absent in the

mesophilic homologous xylanases. Solvent shielding may enhance electrostatic interaction through lowering of the dielectric, and contribute to increased stability of the enzyme.

- L49 ANSWER 33 OF 87 SCISEARCH COPYRIGHT (c) 2008 The Thomson Corporation on STN DUPLICATE 14
- AB Agar-immobilized alkaliphilic Bacillus sp. AR-009 cells were used for xylanase production using batch and continuous culture. In a batch culture, maximum enzyme production was observed after 48 h and remained high up to 72 h. In repeated batch cultivation, immobilized cells produced an appreciable level of xylanase activity in seven consecutive batches without any significant decline in productivity. For continuous xylanase production, immobilized cells were packed in a jacketed glass column and sterile medium was continuously pumped. A stable continuous production of xylanase was observed over a period of 1 mo. The volumetric productivity of the continuous culture was 17-fold higher than the batch culture using free cells.
- L49 ANSWER 34 OF 87 SCISEARCH COPYRIGHT (c) 2008 The Thomson Corporation on STN
- AB An efficient and simple modified method of electroelution is described that can be used as a time-saving method for eluting multiple protein bands. Provided that the proteins are highly expressed, they can be purified rapidly and without requiring any prior knowledge of the protein characteristics. A xylanase excreted by Bacillus sp. CCMI 966 was purified directly from the polyacrylamide gel. Some of the properties of this enzyme are presented. It had an unusually apparent high molecular mass of 340kDa, as determined by native PAGE. The specific activity of the purified xylanase was 137 U/mg. (C) 2000 Elsevier Science Inc. All rights reserved.
- L49 ANSWER 35 OF 87 MEDLINE on STN

DUPLICATE 15

- AB The extracellular xylanase from Bacillus stearothermophilus T-6 is a thermostable alkaline tolerant enzyme that was found to bleach pulp optimally at pH 9 and 65 degrees C, and was successfully used in a large-scale bio-bleaching mill trial. In an attempt to obtain a heavy atom derivative suitable for complete X-ray analysis, xylanase T-6 was labeled biosynthetically with seleno-methionine, resulting in a 'built-in' array of atoms with specific X-ray anomalous scattering signal. Optimization of growth conditions resulted in over 0.8 g of homogeneous seleno-methionine xylanase T-6 per liter culture. The seleno-methionine enzyme was shown to be fully active and produced single crystals suitable for complete multiple wavelength anomalous diffraction (MAD) structural analysis.
- L49 ANSWER 36 OF 87 MEDLINE on STN DUPLICATE 16
- AB Xylanase production of newly isolated thermophilic alkali-tolerant Bacillus sp. strain SP and strain BC was investigated in batch and continuous cultures. Enzyme synthesis was inducible with both strains and was observed only in xylan-containing media. Xylan from oat spelt is a better inducer than xylan from birch for strain Bacillus sp. BC while such difference was not observed for strain SP. Compared with batch cultures xylanase production of both strains increased about two times and its rate became more than four times faster in continuous cultures at a dilution rate of 0.2 h(-1).
- L49 ANSWER 37 OF 87 MEDLINE on STN DUPLICATE 17
- AB A 1.0 kilobase gene fragment from the genomic DNA of an alkaliphilic thermophilic Bacillus was found to code for a functional xylanase (XynII). The complete nucleotide sequence including the structural gene and the 5' and 3' flanking sequences of the xylanase gene have been determined. An open reading frame starting from

ATG initiator codon comprising 402 nucleotides gave a preprotein of 133 amino acids of calculated molecular mass 14.090 kDa. The occurrence of three potential N-glycosylation sites in XynII gene is a unique feature for a gene of bacterial origin. The stop codon was followed by hairpin loop structures indicating the presence of transcription termination signals. The secondary structure analysis of XynII predicted that the polypeptide was primarily formed of beta-sheets. XynII appeared to be a member of family G/11 of xylanases based on its molecular weight and basic pI (8.0). However, sequence homology revealed similar identity with families 10 and 11 of xylanases. The conserved triad (Val-Val-Xaa, where Xaa is Asn or Asp) was identified only in the xylanases from alkaliphilic organisms. Our results implicate for the first time the concept of convergent evolution for XynII and provide a basis for research in evolutionary relationship among the xylanases from alkaliphilic and neutrophilic organisms. Copyright 1999 Academic Press.

- L49 ANSWER 38 OF 87 SCISEARCH COPYRIGHT (c) 2008 The Thomson Corporation on STN DUPLICATE 18
- AB Bacillus sp. AR-009 produced up to 720 U/g dry bacterial bran xylanase activity when grown by using solid-state fermentation with wheat bran serving as a substrate. Xvlanase production was highest at a wheat bran-to-moisture ratio of from 1:0.5 to 1:1.5 and an Na2CO3 concentration of 10% (w/w). Strong repression of xylanase production was observed in the presence of 5% (w/w) xylose and lactose, whereas sucrose and glucose at the same concentration slightly affected enzyme production. The effect of glucose was concentration-dependent, inducing less than 10% of the maximum xylanase production at a concentration of 15% (w/w). No significant effect was observed on xylanase production upon addition of peptone and tryptone, whereas yeast extract slightly stimulated enzyme production. The ability of the organism to produce high-titer xylanase activity at alkaline pH and lower wheat bran-to-moisture ratio could have a potential advantage in minimizing the risk of contamination. In addition, because the enzyme could be extracted by using a minimum volume of liquid, the cost of downstream processing during product upgrading and the cost of waste treatment steps can be greatly reduced. The use of solid-state fermentation for the production of xylanase by Bacillus sp. AR-009 could, therefore, lead to substantial reduction in the overall cost of enzyme production. (C) 1999 Elsevier Science Inc. All rights reserved.
- L49 ANSWER 39 OF 87 BIOSIS COPYRIGHT (c) 2008 The Thomson Corporation on
- L49 ANSWER 40 OF 87 SCISEARCH COPYRIGHT (c) 2008 The Thomson Corporation on STN DUPLICATE 19
- AB Two xylanases, designated XylA and XylB, were purified from the culture supernatant of the alkaliphilic Bacillus sp. strain AR-009. The molecular masses of the two enzymes were estimated to be 23 kDa (XylA) and 48 kDa (XylB) by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The optimum pHs for activity were 9 for XylA and 9 to 10 for XylB. The temperature optima for the activity of XylA were 60 degrees C at pH 9 and 70 degrees C at pH 8. XylB was optimally active at 75 degrees C at pH 9 and 70 degrees C at pH 8. Both enzymes were stable in a broad pH range and showed good stability when incubated at 60 and 65 degrees C in pH 8 and 9 buffers.

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